ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION.

VI. The Action of Acids.

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In recent years the rôle of acidity and alkalinity in biological processes has been the subject of numerous investigations. The impetus to this field of research was given by the invention of methods for determining the true hydrogen ion concentration of biological fluids and materials. As a result of the great mass of work accumulating along this line of research, there prevails among biologists the impression or opinion that hydrogen ion concentration is of tremendous importance in the life of organisms. Yet, looking back upon the history of science, one may be pardoned for a certain degree of skepticism. Biologists appear prone to attach undue significance to whatever field of investigation happens to be the fashion of the decade and time alone can assign a mass of research on one particular topic to its proper place.

The present series of experiments was undertaken in part as a test of the proposition that hydrogen ion concentration is of fundamental importance in physiological processes. The consumption of oxygen in respiration was chosen as a physiological process essential to the organism in the highest degree and readily susceptible to quantitative measurement. An attempt was made to determine: (*a*) whether increasing the acidity of the environment of an organism has any effect upon the rate of oxygen consumption; (*b*) whether this effect is assignable to the free hydrogen ions or to some other factor. The experiments prove that alterations in external acidity markedly affect the rate of respiratory metabolism of animals; but they also show that the free hydrogen ions are little, if at all, responsible for the observed effect.

Literature.

Surprisingly enough, very few investigations have been carried out on the effect of increased acidity on the respiratory metabolism of organisms. A rather careful search through the iterature has revealed only the following researches.

Among plants, only the lower forms have been investigated. Brooks ('21, '22) measured the carbon dioxide production of *Bacillus butyricus*, *B. subtilis*, and *B. tuberculosis* at various hydrogen ion concentrations. The bacteria were tested in dextrose solutions made in distilled water and the desired acidity was obtained with sulphuric acid. The rate of carbon dioxide production was at the maximum at pH 7.0 for *B. butyricus*, at pH 6.8 for *B. subtilis*, and was decreased at all acidities greater than these. In the case of *B. tuberculosis*, however, the carbon dioxide production was constant at all acidities between pH 7.4 and 4.4 and was decreased only at acidities greater than 4.4. Gustafson ('20) studied the effect of altered acidity on the respiration of the mold *Penicillium chrysogenum*. Variations in acidity between pH 8.0 and pH 4.0 were without effect upon the rate of carbon dioxide production. In acidities ranging from pH 4.0 to 2.65, the carbon dioxide output first rose and then fell; below 1.95 an irreversible decrease in the output was observed. The oxygen consumption was measured at pH 2.0 and was found to be markedly increased over the normal, thus agreeing with the initial carbon dioxide production at this particular acidity. In Gustafson's experiments, the mold was tested in dextrose solutions in distilled water and sulphuric and phosphoric acids were employed to increase the acidity.

Among animals, a few researches are available on eggs, tissues, and intact animals. Loeb and Wasteneys ('11) found that the oxygen consumption of sea-urchin eggs is slightly decreased in sea-water acidified to pH 6.0 to 4.0, and considerably decreased at or below pH 4.0. Thunberg ('09, '10) has carried out a large number of experiments on the action of acids on the rate of oxygen consumption and carbon dioxide production of surviving frog's muscle. The tests were performed in salt solution. Thunberg unfortunately did not determine the pH of his solutions—this procedure was not customary at that time—but gives the concentration of acid in terms of molar strength. Hydrochloric acid decreased both oxygen consumption and carbon dioxide output of the muscle markedly, the decrease varying from 15 per cent. in 1/200 mol. HCl to 72 per cent. in 1/20 mol. HCl. Thunberg also tested the action on gaseous exchange of the frog's muscle of a large number of organic acids, including mono-, di-, tri-, and oxycarboxylic acids of both the paraffin and olefin series. Each acid was tested in the following concentrations: 1/100, 1/25, 1/10, and 1/5 molecular strengths. In the majority of the acids, both the oxygen consumption and carbon dioxide production were decreased at all concentrations but not very greatly so. The maximum decrease with the strongest concentrations was 15–25 per cent. In some of the acids (*e.g.*, propionic, butyric, lactic), the respiratory rate was increased in the more dilute concentrations, decreased in the stronger solutions. In fumaric, malic, and citric acids, the carbon dioxide output was greatly increased, while the oxygen consumption was but little altered. On the other hand, oxalic and malonic acids were found to decrease the carbon dioxide output to a much greater extent than the oxygen consumption. Succinic acid alone decreased the former while increasing the latter. It must be remarked that the concentrations used by Thunberg are extremely high and it is difficult for me to believe that the muscle could have remained uninjured in any of the solutions employed by Thunberg, even though each experiment lasted for only thirty minutes. Exposure to 1/50 or 1/20 mol. HCl, for instance, concentrations used by Thunberg, is, I believe, rapidly fatal to any living tissue. Thunberg does not consider the possibility of injury to the muscle in his experiments and makes no statements concerning the condition of the muscle during or after the exposure. I do not believe his experiments can be accepted as of physiological significance. Gray ('24) has studied the action of acids on the oxygen consumption and activity of ciliated tissues of bivalves. Acetic, butyric, and hydrochloric acids were used, presumably in sea-water. In the acidified water, both ciliary activity and oxygen consumption were diminished and in relation to the amount of acidity. Acetic acid at a pH of 4.6 to 4.2 and butyric acid at 4.2 caused a very great decrease and even complete abolition of oxygen consumption. Hydrochloric acid was less effective than either of the two organic acids, producing only 50 per cent. depression at pH 3.0.

There is a dearth of experiments on entire animals. Jewell ('20) tested the carbon dioxide output of frog tadpoles in distilled water acidified with HCl. The output was decreased to 92 per cent. of the normal at pH 7.0, to 84 per cent. at pH 5.4, to 53 per cent. at 3.8, and to 37 per cent. at 3.2. Powers ('23) studied the effect of carbon dioxide tension on the oxygen consumption of the herring. He found the maximum oxygen consumption at pH 7.6 to 7.8; alterations in the carbon dioxide tension, and hence pH, either above or below this value, lead to a decrease, rather slight, however, in the amount of oxygen consumed by the fish. Powers ('22) is of the opinion that the ability of fish to utilize oxygen depends to some extent on the pH of the water in which they are living.

In addition to such direct measurements of the effect of increased acidity of the medium on respiratory exchange there is available a considerable number of researches on the action of acids on processes in which respiratory metabolism is undoubtedly of great importance. Acidification of the medium has a general retarding or depressing effect on such processes. I can refer here only briefly to this literature. It is a well known fact, attested by many researches, that acidification of the medium retards the cleavage and development of the egg and produces abnormal types of larvæ (cf. Loeb, '98, Moore, Roaf, and Whitley, '05, Child, '16, Medes, '17, and Smith and Clowes, '24, for echinoderm eggs; Child, '25, for hydroids; Child, '17, for annelids; Loeb, '98, and Hall, quoted by Shelford, '23, for teleost eggs; and Hall, '24, and Bellamy, '19 and '22, on amphibian development). The retarding effect of acids on regeneration was noted by Jewell ('20). Acidification of the medium constitutes a general method for altering and controlling polarities (cf. Child, '23, and Rustia, '25).

For a general summary of the rôle of acids in the behavior and life of aquatic organisms the excellent paper of Shelford ('23) should be consulted. I am unable however to agree with Shelford that hydrogen ion concentration is of greater importance than carbon dioxide content for aquatic organisms. The facts that Jewell ('22) found an extensive and varied fauna in an acid stream (pH 5.8 to 7.1) and Jewell and Brown ('24), several fishes living in an acid lake (pH 4.4) indicate that hydrogen ion concentration is not of paramount importance in the distribution of aquatic animals. Allee ('23) noted a lack of relation of hydrogen ion concentration to the distribution of marine animals.

From the investigations cited the general conclusion is certainly justifiable that the acidification of the medium to the proper extent retards or depresses biological processes and activities. My own experiments are in agreement with this general result. But without further analysis the conclusion should not be drawn that the observed effect is assignable to the free hydrogen ions of the acidified medium. Natural waters usually contain salts which undergo chemical changes when the water is acidified. In particular, carbon dioxide is evolved. Unless it is definitely proved that the evolution of carbon dioxide or other secondary chemical changes attendant on acidification of natural waters is not concerned in the result, the observed effect should not be assigned to the hydrogen ion concentration *per se.* Even ordinary distilled water contains some carbonates and other salts which may affect the result. Some of the researches cited in the foregoing review cannot be criticized on this score, or only to a slight extent, as distilled water was used, but in other cases, where natural waters were employed, the carbon dioxide evolved was probably of more consequence in the result observed than the hydrogen ion concentration.

In an extensive study of the depressing effect of acidification of natural waters on the oxygen consumption of aquatic animals, presented in this paper, it is shown that in all probability, carbon dioxide is chiefly responsible for the effect.

Methods.

The general course of procedure in the experiments to be reported was as follows. The rate of oxygen consumption of the animals in normal water was determined for a definite period of time, generally one hour. The normal water was then acidified to the desired extent and the oxygen consumption in this acidified water for one or more successive periods of time determined. The animals were placed in Erlenmeyer flasks, if small, or in wide-mouthed salt bottles, if large, of about 500 cc. capacity. The water to be used was thoroughly aërated and placed in a large elevated receptacle. From this it was siphoned into the flasks or bottles containing the animals and allowed to flow out at the top for a few minutes. The flasks or bottles were then tightly closed and kept in a water bath at constant temperature for the desired length of time, generally one hour. After thoroughly shaking the contents, a sample of 125–150 cc. was then drawn by siphon and analyzed for oxygen content by Winkler's method. Blanks of the water used were of course also drawn at the beginning of the experiment and allowed to stand in the water bath with the animals until the end of the test. The difference between the oxygen content of these blanks and the samples drawn from the flasks or bottles containing the animals gives, after suitable calculation, the amount of oxygen consumed by the animals in the time selected. Immediately after drawing the normal samples, the acidified water was added in the same way and the animals allowed to respire in the acidified water for the same length of time. This was repeated as many times as desired, using the same animals and the same degree of acidification in any one series of experiments. The water was freshly acidified and aërated for each of these determinations. There is no possibility that alterations in the oxygen content of the water in any way affected the result, as the oxygen content was at saturation at the beginning of each test, and the test continued only long enough for the animals to use up a small part of the oxygen present.

The hydrogen ion concentration was determined by means of indicators purchased from the La Motte Chemical Products Company. A set of standard tubes covering the range from pH 3.0 to pH 8.4, with indicators brom phenol blue, brom cresol green, brom cresol purple, and phenol red, was employed. Solutions of these indicators were obtained from the same company. A few drops of the appropriate indicator added to 10 cc. of the water to be tested yields a color which can be compared with that of the standard tubes; the approximate pH is thus easily obtained. Brom cresol green was not found to be reliable and consequently pH 5.0 could not be determined very exactly.

The acids employed were: hydrochloric, nitric, sulphuric, carbonic, acetic, butryic, citric, and tartaric. Each of these acids was used in the following concentrations, in terms of pH: 7.5, 7.0, 6.5, 6.0, 5.5, 5.0, and in some cases 4.5. At least three experiments at each concentration of each acid were performed and in many cases a greater number of trials was deemed necessary. It will be seen that the amount of labor involved was very great and this fact must serve as my excuse for any deficiencies in the investigation which may suggest themselves to the reader.

Experiments with Marine Animals.

The experiments recorded in this section were performed in 1922 at the Hopkins Marine Station, Pacific Grove, California. The animals used were two species of starfish, *Leptasterias equalis*, and *Patiria miniata*, and the nudibranch, *Anisodoris nobilis*. It may be noted in passing that starfish and nudibranchs have been found to constitute very favorable material for respiratory experiments.

The animals were placed in wide-mouthed salt bottles and the tests carried out as outlined above. *Leptasterias* is a small species and several individuals were therefore used in each bottle. As the other two species are larger, generally only one individual was necessary. Generally two successive trials of the oxygen consumption in normal sea-water were carried out. The sea-water was then acidified to the desired amount with hydrochloric acid and a determination immediately carried out. The animals were then usually allowed to stand for two to several hours in the acidified sea-water; another test was then performed. The animals then remained in the acid water over night, about 13 to 15 hours in different experiments, whereupon a third test of the respiration in the acidified water was performed. The animals were then returned to normal sea-water, and after several hours the oxygen consumption was again tested in normal sea-water.

It should be understood that for each test freshly aërated and freshly acidified water was used. The water was brought in directly from the ocean as the sea-water coming through the pipes into the laboratory was found to be toxic. In acidifying the water, concentrated HCl was regarded as a 10 mol. solution and sufficient of this was added to a measured quantity of sea-water to give theoretically a certain molecular concentration of hydrochloric acid. Actually, of course, owing to the salts in the sea-water, the concentration obtained is less than that given in the tables. The hydrogen ion concentrations of these solutions could not be determined as I did not have with me the necessary outfit. It may be roughly estimated, however, that 1/1000 HCl in sea-water is about pH 7.0, 1/800 about pH 6.5, 1/600 about pH 6.0, and 1/400 between 5.0 and 5.5. When allowed to stand, of course, such solutions become continuously more alkaline, owing to the escape of carbon dioxide. For this reason, the solution was not made until a few minutes before it was to be used in the tests. The pH of normal sea-water is about 8.2.

The acidification of the sea-water has little or no noticeable effect upon the behavior of the animals in the lower concentrations, 1/1000 mol. or less. In concentrations of 1/800 mol. or greater, the animals are markedly affected. The starfish withdraw their tube feet into the ambulacral groove. The rays are retroflexed towards the aboral surface. In this position the animals remain during the exposure to acid and are apparently unable to move about or to cling with the tube feet. Nudibranchs display a similar behavior. In the stronger solutions they lose their ability to hold to the substratum with the foot and float about in the water in a state of immobility. The gills however remain expanded and it is not believed that differences in the degree of expansion of these organs are to any extent responsible for the depressing effect of acid on the oxygen consumption. In brief, acidification of the water appears to paralyze the neuro-muscular apparatus and to reduce the animal to a state of forced immobility. Recovery is prompt and complete upon return to normal sea-water. None of the concentrations used had any injurious effects upon the animals.

The results of the experiments are given in part in Tables I and II. A typical experiment for each concentration of acid used for one of the starfish species (*Patiria miniata*) and for the nudibranch is presented in Table I. Three trials of each concen-

Table I.

Effect of the Acidification of the Sea-water with Hydrochloric Acid on the Rate of Oxygen Consumption of the Starfish *Patiria miniata*, and the Nudibranch *Anisodoris nobilis*.

Oxygen consumed given in cubic centimeters, per ninety minutes for the starfish, per hour for the nudibranch. Each vertical column constitutes one experiment, showing successive tests of the oxygen consumption of the same animals or animal. The concentration of the acid is given in molecular strengths.

Starfish.				Nudibranch.			
Oxygen Consumption in Normal Sea-water.							
.43	.40	.31	.34	.78	1.02	.68	.83
Oxygen Consumption in Sea-water Acidified with HCl.							
1/1000	1/800	1/600	1/400	1/1500	1/1000	1/800	1/600
Immediate Effect.							
.41	.32	.23	.22	.76	.77	.49	.50
Effect after 2 to 6 Hours' Exposure.							
.35	.31	.21	.22	.74	.78	.57	
Effect after 13 to 15 Hours' Exposure.							
.42	.34			.74	.75		
Recovery on Return to Normal Sea-water.							
.42	.44	.32	.34	.76	.97	.72	
Initial per cent. Depression.							
5	20	26	36	3	25	28	40

Table II.

Average Initial per cent. Depression of All Experiments on Marine Animals.

Conc. HCl.	1/1500	1/1000	1/800	1/600	1/400
Leptasterias		31	38	38	51
Patiria		9	21	28	34
Anisodoris	7	30	26	31	

tration were made but only one of these is given in the table, and may be taken as typical of the three. The results on the other species of starfish (*Leptasterias equalis*) being in no wise different from those on Patiria are omitted for the sake of economy of space. In Table II. is given a summary of all of the experiments, the three trials with each concentration being averaged. The percentage of depression is figured from the oxygen consumption during the first exposure to the acid.

These experiments, although somewhat preliminary in nature, nevertheless exemplify the action of acids on respiratory metabolism. The conclusions which they furnish were verified by later more extensive work. These conclusions may be briefly stated at this point:

1. Acidification of the medium lowers the rate of oxygen consumption of animals.

2. This depression is greater the greater the degree of acidification up to a certain point.

3. Low concentrations are, however, relatively more effective than high concentrations.

4. After a certain degree of acidification has been attained, further acidification does not greatly increase the amount of depression of the oxygen consumption. The maximum depression obtainable is about 50 per cent.

5. The depressing effect of acids on respiratory metabolism is completely reversible, providing the concentration of acid employed is not actually injurious to the tissues of the animal.

Experiments with *Planaria* in Unaltered Fresh Water.

The experiments to be reported in the remainder of the paper were performed at Chicago and occupied the greater part of my time from October, 1923, to February, 1925. The aim of the experiments was to test the effect upon the oxygen consumption of a fresh water animal of the acidification of fresh water with various acids at a variety of hydrogen ion concentrations. The animal selected for this purpose was the flatworm, *Planaria dorotocephala*. It was chosen for a variety of reasons: it is very abundant, is easily kept under laboratory conditions, has no calcareous parts which might be affected by acid, can be kept quiet during respiratory work, and finally had already served as material for a large amount of physiological experiment. I had originally intended to work also with fish and crayfish but the experiments on *Planaria* required a much greater expenditure of time than I had anticipated and I did not feel justified in spending any more time on the matter. I was further informed by Dr. E. B. Powers that he was already engaged in a similar investigation on fish, and I was quite willing to relinquish this particular task to him.

1. *General Procedure.*—There is little to add to the procedure already outlined. Large stocks of *Planaria dorotocephala* are always at hand in our laboratory. Only stocks which had sojourned in the laboratory for at least one or two months were employed, as the basal respiratory rate of freshly collected material is variable. Only worms which had starved from four days to two weeks were used, since during this period the respiratory rate is constant. Each lot of worms to be used for an experiment consisted of a number of individuals sufficient to consume a readily measurable amount of oxygen in an hour. Such a lot was placed in a 500 cc. Erlenmeyer flask at least several hours before the experiment because when the worms are placed in any clean strange container they are apt to travel about restlessly for some time. By placing the worms in advance in the container in which they are to be tested and by darkening them during the experiment, movement can be practically entirely eliminated. It is certain that movement does not play any rôle in the results. Three such lots of worms were carried throughout the work in order that three tests of each strength of each acid could be performed simultaneously. This procedure shortens considerably the amount of labor involved. Each lot of worms was kept in a particular flask for nearly two weeks and used several times during that period. If desired such lots could then be fed once or twice in the flasks and after four days again utilized for experiments. This procedure was found most suitable because different lots of worms are apt to yield different percentages of depression with the same concentration of the same acid. To obtain a graded set of results showing a graded increase in the percentage of depression with increasing concentration of acid, it is almost necessary to test all of the concentrations of any one acid on the same lot of worms. If different lots are interpolated in such a series, the relation between depression and concentration is less regular, although always exhibiting the same general trend.

The remainder of the procedure was as already described. It may be repeated once more that for each hour's test freshly aërated water was employed and this was freshly acidified to the desired degree a few minutes before being used for each hour's determination. Each test lasted for one hour. A determination of the normal rate of oxygen consumption in normal water (pH 8.0) was first run, and this was followed without pause by a determination in the acidified water. In many cases there were two or three trials in the acidified water, usually with intervals between the trials. During such intervals the animals remained of course in the acidified water. As it was found by repeated experiment that the maximum percentage of depression is obtained on the initial exposure to the acid, these later tests in acid were omitted in the latter part of the investigation. Generally the investigation of each acid was begun with the lowest concentration (pH 7.5) and the concentration increased by steps of 0.5 pH at each succeeding experiment. As will be discussed later this procedure gives the most regular results and great irregularities are introduced if the strongest concentrations are employed first.

There is not the slightest possibility that any differences in the oxygen content of the water either at the start of each test or produced during the test by the withdrawal from the water of oxygen by the animals in any way affects the results. The oxygen consumption of *Planaria dorotocephala* is entirely independent of the oxygen content of the water at all concentrations between 8 and 2 cc. per liter. Whether it is affected by concentrations above or below these limits has not been determined. Suffice it to say that in none of the experiments here recorded nor in any that I have reported in the past with this species has the oxygen content of the water ever reached a value which could have affected to the slightest degree the rate of oxygen consumption of the animals.

The acids used and the hydrogen ion concentrations at which they were tested have already been stated. Concentrations which might have injured the animals during the exposure were avoided. For this reason very few tests were run at acidities greater than pH 5.0. It was desired to keep within physiological concentrations.

All of the experiments on *Planaria* were performed at a temperature of 20° C. and the oxygen in all cases is calculated for this temperature. The results are given in cubic centimeters of oxygen consumed per hour. The animals were not weighed as the oxygen consumed per gram is of no importance for the present experiments. For the benefit of those who may be curious regarding this matter it may be stated that the normal rate of oxygen consumption of this species under the conditions of the experiments (worms of medium size, four to fourteen days after feeding, temperature 20° C.) is about .20 cc. of oxygen per gram per hour.

2. *Water.*—The water used in the experiments comes from a well driven into the ground from the floor of the basement of the laboratory. This water when it emerges from the taps is supersaturated with air and also contains a considerable amount of free carbon dioxide. It has a pH of about 7.3. On account of the gas content the water is heated but not boiled and then allowed to cool overnight before being used for experimental purposes. Water so treated has a pH of 8.0 to 8.2. This is the water regarded in the experiments as "normal" and the respiration in such water is taken as "normal." The water is of course thoroughly aërated before use, bringing the oxygen content up to 6 to 8 cc. per liter.

Table III.

Analysis of the Water Used in the Experiments on *Planaria*.

All figures represent parts per million. Analysis made in 1922.

Determinations as		Hypothetical combinations.	
Iron—Fe	0.2	Potassium nitrate	1.4
Manganese—Mn	0.1	Potassium chloride	1.1
Silica—SiO_2	7.8	Sodium chloride	7.6
Nonvolatile	0.7	Ammonium chloride	0.1
Alumina—Al_2O_3	1.6	Magnesium chloride	1.0
Calcium—Ca	49.2	Magnesium sulphate	22.1
Magnesium—Mg	16.7	Magnesium carbonate	41.5
Ammonia—NH_4	0.03	Calcium carbonate	123.0
Sodium—Na	3.0	Silica—SiO_2	7.8
Potassium—K	1.1	Alumina—Al_2O_3	1.6
Sulphate—SO_4	17.7	Iron oxide—Fe_2O_3	0.3
Nitrate—NO_3	0.9		
Chloride—Cl	6.0		
Alkalinity (methyl orange)	170		
Residue	193		

The water used is thus the ground water of the Chicago region. It is high in salt content, particularly in carbonates. This water has been analyzed for us by the State Water Survey Division of Illinois. It seems desirable to present the analysis at this point.

3. *Behavior of Planaria in Acid.*—When the water is acidified no alteration in the behavior of the worms is noticeable until a certain degree of acidification is reached. This degree is different for different acids, but is generally in the neighborhood of pH 5.5 to 5.0. The animals lose the ability to glide about, owing apparently to a paralysis of the ciliary mechanism. They exhibit continuous writhing movements, and secrete a large amount of mucus. With greater acidification, they lose ability to cling to the glass and fall to the bottom of the container, where they remain, often with slight writhing movements. They are, when this condition is attained, extremely elongated and generally more or less curved, the ventral surface being concave, the dorsal convex. In short, as in the case of the marine animals, a sufficient degree of acidity induces a paralysis of the motor mechanism, which appears to involve the cilia first, the muscles later.

4. *Experiments with Hydrochloric Acid.*—A large number of experiments, about seventy, were performed with this acid. The normal respiration in normal water (pH 8.0) for one hour was first determined. The water was then acidified to the desired degree and the rate of oxygen consumption in the acid water immediately tested. An interval of one hour in the acidified water was then passed, another determination made in freshly acidified water, another hour passed, and a third determination carried out. It will thus be evident that the respiration during the first, third, and fifth hours in the acid was tested. This was the general procedure in the earlier part of the work. The acidity ranged from pH 7.5 to 4.5 at 0.5 pH intervals. It seems unnecessary to present in detail the mass of data thus accumulated as all of the experiments gave the same general result. A set of typical determinations of the action of hydrochloric acid at different hydrogen ion concentrations is given in Table IV. The averages of all of the experiments performed with this acid are summarized in Table VI. The action is graphed in Fig. 1.

Table IV.

Action of Hydrochloric and Sulphuric Acids at Different Hydrogen Ion Concentrations on the Oxygen Consumption of *Planaria*.

All figures represent cc. of oxygen consumed per hour at 20° C. Each vertical column constitutes one experiment, giving successive determinations on the same lot of worms.

Normal Respiration, pH 8.0.													
	.42	.44	.37	.48	.34	.34	.42	.43	.38	.40	.50	.58	.43
Respiration in Acidified Water.													
	Hydrochloric.							Sulphuric.					
pH	7.5	7.0	6.5	6.0	5.5	5.0	4.5	7.5	7.0	6.5	6.0	5.5	5.0
1st hr.	.36	.34	.28	.34	.23	.20	.23	.36	.27	.27	.33	.37	.24
3d hr.	.38	.37	.33	.37	.24	.22	.25	.38	.30	.34	.39		
5th hr.	.38	.33	.30	.38	.27	.20		.34	.34	.26	.43		
Per cent. Initial Depression.													
	13	23	25	29	33	40	46	16	29	35	34	37	45
Recovery after Return to Normal Water.													
	.44	.41	.41	.41	.34	.32	.35	.49	.39	.41	.44	.49	.41

Fig. 1. Graph of the action of acids on the rate of oxygen consumption. Curve *A*, action of hydrochloric acid in carbonate-free water. Curve *B*, action of hydrochloric acid in ordinary water. Dashed line, average curve of the depressing action, obtained by averaging all of the acids except butyric. Percentage of depression, normal respiration being taken as 100, on the ordinate, pH on the abscissa.

It will be perceived by examining these tables that the rate of oxygen consumption of *Planaria* is decreased by acidifying the water with hydrochloric acid and that the percentage of depression is greater the higher the acidity. Yet it must be remarked at this point that the percentage of depression obtained with any given concentration of acid is subject to inexplicable variation even on the same lot of worms and quite commonly when different lots of worms are employed. In most cases this variation did not exceed 10 per cent. but it may reach 20 to 30 per cent., in the higher concentrations. On account of these puzzling variations I was frequently compelled to repeat the experiments many more times than had been anticipated as necessary. The matter of variation is considered at more length later and a possible cause is suggested.

Table V.

Action of Carbonic and Acetic Acids at Different Hydrogen Ion Concentrations on the Oxygen Consumption of *Planaria*.

All figures represent cc. of oxygen consumed per hour at 20° C. Each vertical column constitutes one experiment, giving successive determinations on the same lot of worms.

Normal Respiration, pH 8.0.												
	.34	.21	.31	.29	.28	.25	.40	.50	.50	.36	.51	.71
Respiration in Acidified Water.												
	Acetic Acid.						Carbonic Acid.					
pH	7.5	7.0	6.5	6.0	5.5	5.0	7.5	7.0	6.5	6.0	5.5	5.2
1st hr.	.30	.26	.28	.24	.20	.14	.44	.46	.39	.27	.35	.43
3d hr.	.36	.26	.37	.28	.25	.14		.45		.25		
5th hr.	.31		.32									
Per cent. Initial Depression.												
	12	+23	10	18	29	44	+10	8	22	25	32	40
Recovery after Return to Normal Water.												
	.36	.20	.29	.28	.31		.41	.50	.46	.44	.52	

5. *Experiments with Sulphuric and Nitric Acids.*—The action of these two mineral acids was very similar to that of hydrochloric acid. The percentage of depression induced was consistently slightly greater at the same hydrogen ion concentrations

than that produced by HCl. Some detailed data on sulphuric acid are given in Table IV. and the results of all experiments with nitric and sulphuric acids are summarized in the general table, Table VI.

Table VI.

Summary of All Experiments with All Acids, giving the Average Percentage of Depression on Initial Exposure to Each Acid at Each pH Used.

pH.	7.5	7.0	6.5	6.0	5.5	5.0	4.5	3.8
Hydrochloric	14	17	26	26	29	35	45	36
Sulphuric	21	22	27	33	33	34		
Nitric	18	26	28	30	40	40		
Carbonic	+ 2	3	20	21	31	45		
Acetic	6	+ 8	10	31	30	40[1]		
Butyric	16	7	15	7	8	37[1]		
Tartaric	13	14	22	21	31	33		
Citric	+ 4	10	18	31	40	29		

6. *Experiments with Carbonic Acid.*—Carbon dioxide gas was passed through the water until the desired acidity was obtained. In this procedure great care was necessary to avoid depriving the water of its oxygen, since when a gas bubbles through a liquid it removes other gases. The water had to be vigorously shaken with air after the carbon dioxide had been added. A good deal of manipulation is required when working with higher concentrations of carbon dioxide to secure the proper amount of oxygen and carbon dioxide simultaneously in the water. In all of the experiments reported the oxygen content of the water was ample. It may be noted in passing that a combination of low oxygen with high carbon dioxide has a much greater depressing effect than either of these conditions separately. Under ordinary conditions the rate of oxygen consumption is normal in water containing 2 cc. per liter but oxygen consumption is almost abolished in such water if it be saturated with carbon dioxide. When plenty of oxygen is present, the depression produced by saturated carbon dioxide is only about 50 per cent.

Most of the experiments with carbon dioxide are reported in Table V. In the more dilute solutions—pH 7.5 to 7.0—carbon dioxide acidity produces less depression than do other mineral

[1] Animals injured.

acids. In fact, in these concentrations, there is a tendency towards stimulation of the respiratory rate. Out of nine experiments, stimulations of 2 to 12 per cent. were obtained in five cases. The amount of stimulation, however, lies within the experimental variation. The truth probably is that carbon dioxide acidity does not affect the rate of oxygen consumption until the acidity reaches a greater degree than pH 7.0. At greater acidities than pH 7.0, the rate of oxygen consumption is depressed and to a degree similar to that produced by other mineral acids. It was not possible to obtain an acidity with carbon dioxide greater than pH 5.2 with the well water, owing probably to the buffers present in the water.

7. *Experiments with Acetic and Butyric Acids.*—These two acids were selected as examples of the paraffin acids, supposed to penetrate organisms readily. It was anticipated that the depressing effect of these acids would be greater than that of mineral acids. Such, however, was not the case, but the contrary result appeared. Acetic acid, in acidities between 7.8 and 7.0 tended to stimulate the rate of oxygen consumption. Of six experiments performed at pH 7.5, the rate of oxygen consumption was unaffected in two cases, stimulated in two, and depressed in the remaining two. Of six experiments at pH 7.0, all but one were stimulating. It seems probable that low concentrations of acetic acid accelerate the rate of oxygen consumption. At concentrations greater than pH 7.0, all concentrations depressed the rate of oxygen consumption during the first hour's exposure. During later exposures, a tendency toward acceleration was again manifest. These facts are brought out in Table V. and VI. Butyric acid was the least effective in inducing depression of all of the acids used. This result was very surprising and still remains inexplicable. Butyric acid has only a slight depressing action at all concentrations between pH 7.5 and 5.5. At pH 5.0 a depression of 30 to 40 per cent. appears but this concentration of butyric acid is lethal and the worms begin to die within an hour. Acetic acid at pH 5.0 is also injurious. The data on butyric acid are summarized in Table VI., which gives the initial depression induced. Later exposures did not alter the result.

8. *Experiments with Tartaric and Citric Acids.*—The former was

selected as an example of a dibasic, the latter of a tribasic acid. The results were not of particular interest and are summarized in the general table, Table VI. The action of tartaric acid was very similar to that of the mineral acids. Citric acid was less effective than the inorganic acids at concentrations lower than pH 7.0.

9. *Consideration of Other Factors.*—(*a*) *Size.* It was found long ago by Child that young (small) individuals are more susceptible to lethal concentrations of various substances than are old (large) individuals of a given species. MacArthur ('20) studied the relation between age (size) and susceptibility to acids in *Planaria dorotocephala.* He found that in concentrations of hydrochloric and other acids which are quickly lethal, between pH 2.0 and 4.5, the young (small) individuals die sooner than the old (large) ones. At slightly lower concentrations, pH 4.7 to 4.9 for HCl, the relation is reversed, the larger individuals succumbing first. In still weaker solutions, planarians live indefinitely. These results have been repeatedly confirmed in this laboratory in class work, although of course the precise concentrations required to yield the results mentioned are subject to variation, owing chiefly to differences in the physiological condition of the worms themselves.

Since according to these results, small worms are more susceptible to acids than large worms at concentrations greater than pH 4.5, it seems probable that the percentage of depression induced by acids should bear some relation to size. This was tested in a number of experiments, in which the depressing action of HCl on large and on small worms was compared. Only recently collected material was used as the metabolic differences between worms of different sizes are greater in such material. The heads of all worms were removed before the test, in order to eliminate movement, as small worms are more active than large ones. Decapitated worms are very inactive. The small worms used were under 10 mm. in length, the large ones over 20 mm. Twelve experiments were performed at concentrations of HCl ranging from pH 3.6 to 4.6. In ten of these the percentage of depression was greater in the small than in the large worms. The difference ranged from 3 to 20 per cent. In two cases the result was reversed, the larger worms exhibiting about 10 per cent. more depression than the small ones. In general, then, the experiments indicate that in concentrations which will kill within a few hours and in which small worms die slightly faster than large worms, the small worms are more greatly depressed by the acid. It should be added that the animals suffered no injury during the period of exposure and recovered completely.

(*b*) *Previous History with Respect to Acid.*—From my experiments I have gained the distinct impression that the amount of depression induced by exposure to acidified water is to some extent dependent on the previous history with respect to acid. If a given lot of worms, which has never been exposed to acidified water, is tested at rather high acidity, say pH 5.0, it is commonly found that the amount of depression is much less than would ordinarily be expected at that acidity. If on the other hand, such worms are gradually accustomed to acidified water, by exposing them first to pH 7.5 and gradually increasing the acidity, then a much greater percentage of depression is obtained at high acidities. One of the most striking cases of this kind in my investigation occurred in working with sulphuric acid. Three lots of worms, never before exposed to acidified water, were tested in water acidified to pH 5.0 with sulphuric acid. The percentages of depression obtained were 20, 30, and 15 respectively. The same lots of worms were then exposed on successive days to various concentrations of sulphuric and hydrochloric acid, beginning at 7.5 and gradually working down to pH 5.0 again. When sulphuric acid pH 5.0 was then again tried, on the same worms, the depressions obtained were respectively 39, 56, and 45 per cent. The same type of result was frequently met with. It appears that when worms are suddenly exposed to rather strongly acidified water, they are able to produce or manufacture some substance which protects them from the acid. But after repeated exposures to acidified water, the substance is either exhausted or the worms have become so accustomed to residing in acidified water that they no longer respond to it by producing the substance. Sudden exposure to acid may act as a stimulus to production of basic substances; repeated exposure fails to stimulate.

It seems probable that in this behavior of the worms is to be found the explanation of the numerous puzzling variations in the degree of depression obtained.

(*c*) *Acclimation.*—It was my intention at the beginning of this work to determine whether the animals could recover from the depression induced by acid if allowed to remain for some time in the acidified solution. I found, however, that the experiment is impractical, because of the carbonate content of the water used. When the water is acidified it soon becomes alkaline and unless acidified to a point which would be fatal to the worms, returns to an alkaline condition within 24 hours. Consequently if worms are placed in the acidified water at a certain pH, the water does not remain at this pH, but the pH rises (the acidity falls). If then the worms are tested at the new pH, the depression is naturally less than it was at the beginning of the exposure. If tested at the original pH, the depression is greater than at the pH attained by the standing solution. In brief, it is impossible to determine the effect of long continued exposure to a given pH, unless the water is freshly acidified and changed every hour, for two or three days. As this is physically impossible, for me at least, the experiment had to be abandoned. I have, however, a number of experiments, especially with HCl, in which the oxygen consumption was tested during the first, third, and fifth hours of continuous exposure to a given concentration. Some of these data appear in Table IV. In general it was found that there is very slight if any recovery during successive hours of exposure. After this had been repeatedly determined, the tests of the later exposures were abandoned.

The reader will no doubt at once inquire why carbonate-free water was not employed in a study of acclimation. The reason for this as will appear later is that acidified carbonate-free water does not affect the oxygen consumption of Planaria.

10. *General Results and Discussion.*—We may now state and discuss the results obtained with acidified unaltered water.

(*a*) Acidification of the water generally causes a depression of the rate of oxygen consumption. This was true of the three mineral acids, hydrochloric, sulphuric, and nitric, at all concentrations employed. Even a change from the normal pH 8.0 of the water to 7.8 by addition of such acids induces a measurable lowering of the rate of oxygen consumption. In the case of some acids, notably acetic, less so with carbonic, there was some tendency to an acceleration or stimulation of the oxygen consumption at acidities between 7.8 and 7.0. Yet this effect was so slight, and the variability of the results so great that little emphasis can be placed upon this finding, unless a very large number of experiments were carried out. All of the acids used caused depression at all acidities greater than pH 7.0, but the action of butyric was very slight, almost nil.

(*b*) The percentage of depression increases with increasing acidity within certain limits.

(*c*) The lower concentrations are, however, relatively more effective than the higher concentrations. This is generally the case wherever chemicals are applied to living organisms. The general form of the curve obtained is seen in Fig. 1. This type of curve is so commonly obtained in physiological experimentation that it must possess some deep significance. I am unable however to suggest any explanation of this type of curve.

(*d*) The inorganic acids, except carbonic, are in general more effective than the organic, particularly in the lower concentrations, pH 7.5 to 6.5. This finding was the contrary of my expectations and contrary to the results of Gray ('24). It is generally believed that organic acids, such as acetic and butyric, penetrate protoplasm readily, while mineral acids are unable to do so. Obviously one would then expect the organic acids to act more powerfully on respiratory metabolism than the inorganic acids. As this was not found to be the case, it must be concluded that the penetrability of the acid has no bearing on the result. The lack of action of butyric acid remains inexplicable.

(*e*) The hydrogen ion concentration cannot be the chief cause of the depression induced, because different acids do not produce the same percentage of depression at the same pH. The result does not substantiate the contentions of Loeb ('22) that the action of acids on colloids depends only on valence and hydrogen ion concentration. According to Loeb's ideas, all of the monovalent acids, such as hydrochloric, nitric, acetic, and butyric should have produced the same amount of depression at the same hydrogen ion concentration. Reference to Table VI. shows that this is not at all the case, butyric acid furnishing a notable exception. According to Loeb also, di- and tri-basic acids should be less effective than monobasic. This again is not upheld by my results. Sulphuric acid is even more effective than hydrochloric, although being dibasic it should be only half as effective. Tartaric, another dibasic acid, has about the same efficiency as the monobasic mineral acids. Of course, it is not certain that Loeb intended his ideas to apply to living organisms. Further the hydrogen ion concentrations with which Loeb worked are mostly instantly or rapidly fatal to living organisms. It remains to be proved whether the statements of Loeb will hold at physiological concentrations of acid. I have been informed that Michaelis has publicly stated that they do not hold and that the Hofmeister series remains unshaken. From the fact that in my experiments different acids produce different degrees of depression at the same hydrogen ion concentration it appears necessary to conclude that the hydrogen ion concentration is not the principal factor in the result.

(*f*) When a certain degree of acidification has been attained, further acidification does not increase the percentage of depression. The percentage of depression obtained at pH 5.0 is about the maximum that can be produced without actual injury to the animals. It appears that a depression of 50 per cent. is the most that can be obtained with any acid on the average. Of course individual experiments may yield a depression slightly greater than this. A depression of 58 per cent. is the greatest recorded in the dozens of experiments performed with the various acids and this figure was obtained on young worms. The statements in this paragraph apply only when an ample supply of oxygen is present in the water.

(*g*) The decrease in the rate of oxygen consumption reaches its maximum value for any particular concentration during the first hour of exposure to the acidified water. Prolonged exposure does not increase the depressing effect. On the contrary there is generally some slight rise in the oxygen consumption during several hours exposure. This is too small however to be considered of significance.

(*h*) The depressing action of acids is completely and promptly reversible, wherever an actual injury to the tissues of the animal has been avoided. Recovery occurs almost immediately, commonly within the first hour after return to normal water. Several experiments were devised to test the possibility that the decreased oxygen consumption while in the acid might be compensated for by an increase over the normal during the period immediately upon return to normal water. In these experiments the oxygen consumption was first tested in normal water, then in acidified water, of a concentration to give at least 30 per cent. depression, then immediately in normal water again. In most of these cases the respiration had risen to the normal value during the first hour after return to normal water. In a few cases, the oxygen consumption was below normal. In no case was any rise over the normal figure observed.

(*i*) The experiments justify the use of acids as agents for experimentally producing a state of depression. They also substantiate the generally held view that the effects produced by acids on such processes as cleavage, development, and regeneration are assignable to a reduction in the rate of respiratory metabolism.

Experiments with *Planaria* in Carbonate-free Fresh Water.

The experiments had reached the point outlined above by the summer of 1924 and I intended to bring the investigation to a close. A number of matters puzzled me greatly but I was unable to devise any means of throwing further light on them. At about this time, however, my attention was drawn to the experiments of Clowes and his associates ('23, '24) in which it was shown that the carbon dioxide set free by acidification of sea-water markedly influences the result and is in some cases the real agent involved. At first I was not inclined to believe that carbon dioxide was responsible for the results which I had obtained with acids. It seemed to me that if carbon dioxide were chiefly or wholly responsible for the observed effects, carbon dioxide acidity should be more effective than acidities produced by other acids and the action of various acids should be similar at the same hydrogen ion concentrations. As these conditions did not obtain in my experiments, I considered it unlikely that the findings of Clowes applied to them. It seemed necessary to me, however, that the matter should be tested by experiment. A new series of experiments was therefore begun in the fall of 1924 using carbonate-free water. The results obtained convinced me that the depressing action of acidified water is largely due to the carbon dioxide liberated in it.

In connection with these experiments a large number of trials in ordinary carbonate-containing water were carried out as controls. These constituted repetitions of the work of the previous year. It was very puzzling to me to find that all acids were less effective than had been the case in the preceding year. This was particularly noticeable at the lower concentrations, pH 7.5 to 7.0. The mineral acids produced about the same depressing effect as previously at the higher concentrations, 6.5 to 5.0. The organic acids (acetic and butyric were the only ones tried) were also markedly less effective than had previously been the case, at all concentrations tried. I am unable to explain this state of affairs except on the assumption that the carbonate content of the water had decreased in the meantime. The fact that in many cases the controls gave a smaller percentage of depression than previously made it difficult to arrive at conclusive results.

1. *Preparation of Carbonate-free Water.*—Carbonate-free water was prepared according to the method of Smith and Clowes ('24*b*). Two cc. of concentrated hydrochloric acid were added to eight liters of the well water in a large bottle. Air from the compressed air system was passed through the water in the bottle for 24 hours or more. This treatment was found to remove the carbonate from the water completely. Several times liter samples of this water were evaporated to dryness and the residue tested for carbonate with entirely negative results. In some cases borax was added to the carbonate-free water to serve as buffer but as the results were not affected by this procedure, it was generally omitted. The oxygen consumption of the worms in this carbonate-free water, with or without borax, was repeatedly compared with that in the normal unaltered well water. No difference was found.

2. *Action of Mineral Acids Added to Carbonate-free Water on the Rate of Oxygen Consumption.*—The same amount of acid was added to the water as would be required to produce the desired pH in unaltered water. Alkali was then added until the pH in question had been attained. Rather extensive experiments were performed with hydrochloric acid, less extensive with sulphuric and nitric acids. The results in all three acids were the same and were very striking. Acidification of carbonate-free water with these acids has *absolutely no effect* on the rate of oxygen consumption of Planaria at all concentrations between pH 7.5 and 5.0. At pH 4.0, a slight depressing effect was noted, about 15 per cent. This result is graphed in Fig. 1.

It is thus proved that practically the entire depressing effect on the oxygen consumption of *Planaria* produced by the addition of mineral acids to natural water arises from the carbon dioxide set free in such water by decomposition of its contained carbonates by the acid added. The depressing effect is thus a carbon dioxide depression. It is probable that the carbon dioxide penetrates the animal in the gaseous state and acts within the animal as such or by inducing an internal acidity (cf. Jacobs, '20).

3. *Action of Organic Acids in Carbonate-free Water.*—Only acetic and butyric were tried. I was particularly interested in testing these two acids as they are believed to penetrate organisms readily. The result should serve to indicate whether the efficiency of carbon dioxide is merely a question of penetrability. Unfortunately decisive results could not be secured, owing to the fact, already mentioned, that very little depressing effect was obtained in the controls. Butyric acid, previously found to be the least effective of all of the acids tried, was in this series of experiments quite ineffective in normal water. Even at pH 5.0, an injurious concentration, no depression of the oxygen consumption in normal water appeared. Consequently the action of butyric acid in carbonate-free water could not be determined. With acetic acid, some results were secured. Acetic acid was about half as effective in inducing depression as in the experiments of the preceding year. At concentrations weaker than pH 7.0 there were again indications of acceleration of the rate of oxygen consumption in the controls. At acidities of pH 6.5

or greater, depression was produced in normal water, but to a less extent than previously. In the same worms, in carbonate-free water acidified with acetic acid, the depression induced was noticeably less than in the controls. It therefore follows that the depressing action of acetic acid is also in large part due to the carbon dioxide which is sets free. It appears that the penetrating powers of carbon dioxide do not entirely explain its difference from other acids.

4. *Action of Carbon Dioxide Added to Carbonate-free Water.*—These experiments were designed as a sort of crucial test of the proposition that carbon dioxide is the cause of the depression induced by acidification of natural waters. The proposition was upheld in the most striking manner. The addition of carbon dioxide gas to the same pH causes the same degree of depression, whether added to normal, or to carbonate-free water. It thus appears to be reasonably certain that the depression of the rate of oxygen consumption in acidified water is caused chiefly by carbon dioxide.

The question then arises: Is the amount of carbon dioxide liberated in carbonate-containing water by the addition thereto of acids, different with different acids at the same hydrogen ion concentration? It became necessary to determine the actual amount of free carbon dioxide present when the well water was acidified with various acids.

5. *Determinations of the Amount of Carbon Dioxide Liberated on Acidification of the Unaltered Water.*—The normal well water, pH 8.0, was acidified to the desired pH with various acids. A sample of 100 cc. was then immediately drawn and the free carbon dioxide in it determined by titration with $N/50$ barium hydroxide, properly protected from the carbon dioxide of the air, using phenolphthalein as indicator.

Hydrochloric, sulphuric, acetic, and butyric acids were investigated at ranges of pH 7.5 to 4.0. It was found that the amounts of carbon dioxide liberated from the carbonates of the water by different acids are similar, but not identical, at the same pH. Such differences probably account for some of the different percentages of depression obtained with different acids at the same pH. But butyric acid was also found to liberate nearly as much carbon dioxide at the same pH as does hydrochloric acid. The lack of depressing action of butyric acid therefore remains at present inexplicable.

Nearly all of the carbonate of the water is liberated as carbon dioxide at a pH of 5.0 and all of it between pH 5.0 and 4.0. The amount of free carbon dioxide present at these acidities is about 30 cc. per liter. At first the fact that all of the available carbonate in the water is decomposed at pH 5.0 to 4.0 appeared to explain the result that the maximum percentage of depression is obtained at such acidity and further acidification does not increase the depression. But the additional fact discussed in the next paragraph that increasing the amount of carbon dioxide by adding the gas is not effective in intensifying the depression invalidates the suggested explanation.

Analyses were also made of the amount of carbon dioxide gas required to produce a certain pH. It was found that more of the gas is present in the water at a given pH than is set free in the water by adding other acids to the same pH. This result would be expected, since it is highly probable that when the water is acidified, considerable time must elapse before all of the acid reacts with the carbonate of the water. Consequently immediately after acidification part of the free hydrogen ions present are derived from the acid added, and not from the reaction of carbon dioxide with water. The difference in carbon dioxide content between water acidified with carbon dioxide gas and water acidified by other acids is small at pH 7.0 or even 6.5 but at pH 6.0 there is three times and at pH 5.0 nearly ten times as much carbon dioxide gas in the water as is liberated by mineral acids at those same hydrogen ion concentrations. These results are shown in Fig. 2.

We are thus faced with the question—why is carbon dioxide not more effective as a depressing agent than any other acid, particularly at the higher acidities? There was to be sure evidence that carbon dioxide at pH 5.0 for instance has a greater depressing action than any other acid tried. Yet the difference between it and other acids is not as great as might be expected. It becomes necessary to assume, in view of the facts at hand, that the amount of depression of the respiratory rate which can be induced in Planaria by carbon dioxide is limited and does not exceed 50 per cent. as long as the oxygen supply remains ample. If the oxygen supply is reduced, although not to a point where the respiration rate would be affected under ordinary conditions,

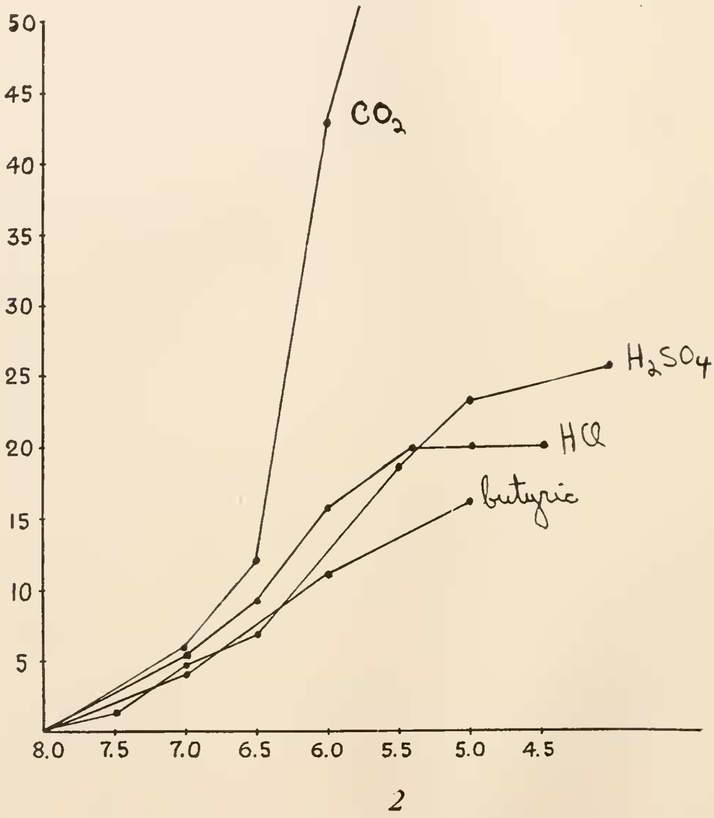

FIG. 2. Graph of the amount of carbon dioxide liberated in the well water by various acids. Amount of carbon dioxide in cc. per liter on the ordinate, pH on the abscissa. The amount of carbon dioxide gas required to produce a given pH is also shown.

the oxygen consumption can be nearly abolished if the water is saturated or nearly so with carbon dioxide. A carbon dioxide content of 30 cc. per liter appears to yield the maximum depressing effect, about 50 per cent., that can be obtained with carbon dioxide, when the oxygen supply is adequate.

6. *Conclusions Concerning the Experiments with Carbonate-free Water.*—(*a*) The depressing effect of acids on the oxygen consumption of Planaria is almost wholly abolished when carbonate-free water is used, except when the acidity is produced by carbon dioxide gas.

(*b*) An acidity due to carbon dioxide gas is equally depressing in ordinary and in carbonate-free water.

(*c*) From the statements in (*a*) and (*b*) it is concluded that the depressing action of acids on oxygen consumption is almost wholly due to the carbon dioxide liberated on acidification of natural waters.

(*d*) Different acids at the same hydrogen ion concentration do not immediately liberate the same amounts of carbon dioxide from the natural carbonate-containing water. This accounts in large part for the fact that the depressing action of different acids is not the same in degree at the same pH.

(*e*) All of the carbonate of the water used is decomposed at a pH of 4.0 to 5.0, giving a carbon dioxide content of about 3 per cent.

(*f*) The lack of action of butyric acid is not explained, for this acid also liberates carbon dioxide from the carbonates of the water. Possibly butyric acid in some way prevents the penetration of carbon dioxide into the animals.

(*g*) The amount of carbon dioxide gas required to produce a given pH is considerably greater at acidities of more than pH 6.5 than is the amount of carbon dioxide liberated in the water by other acids at the same pH. One would therefore expect that CO_2 would be much more effective as a depressing agent at acidities from 6.5 to 5.0 than any other acids. This was not noticeably the case. The difficulty could be explained by assuming that the depressing action of CO_2 reaches a maximum at a concentration of 3 per cent. and is not altered by further increase in the concentration of the gas.

(*h*) Since the depressing effect of acids is due largely to carbon dioxide and since this gas is an end product of metabolism, it becomes readily understood that organisms or parts must be affected by acids in relation to their respiratory metabolism. The higher the rate of the latter, the greater would be the depression induced by acids.

Relative Toxicity of Different Acids.

An attempt was made to discover the hydrogen ion concentration at which each of the acids used would cause the death of *Planaria* within a relatively short period of time, say, two to three hours. A sort of trial and error procedure is necessary to determine this matter. The results are not very exact but the relative toxicities of the different acids were plain enough after a few trials. Butyric acid is by far the most toxic, as found also by other investigators, and kills the animals within two or three hours at pH 5.0. Acetic acid ranks next in toxicity, killing in the time specified at pH 4.4. Tartaric and citric acids come next, killing within two or three hours at 3.6 and 3.4 respectively. The three mineral acids are nearly equally toxic, sulphuric being slightly more effective, killing in two or three hours at pH 3.2 to 3.4, while hydrochloric and nitric acids must be used at pH 3.0 to 3.2 to obtain the same result. The order of toxicity may then be expressed as follows: butyric > acetic > tartaric > citric > sulphuric > nitric = hydrochloric.

Carbon dioxide at saturation (about pH 5.2) was not found to kill the animals as long as the oxygen supply was adequate.

The death of the animals took place with equal rapidity and at the same hydrogen ion concentrations in both normal and carbonate-free water. This proves that the death is not due to the carbon dioxide set free by acids in normal water but is a direct acid effect. This is further evidenced by differences in the appearance and in the manner of disintegration of the dead animals. Death in acids appears to be generally due to coagulation. This was also noted by Mrs. Lewis, '23.

In view of the fact that the death of the animals results from a direct coagulating action of the acids while the depression of the respiratory rate by acids is caused almost wholly by carbon dioxide, no relation would be expected between the toxicity of different acids and their depressing action. This is the case. Butyric acid, the most toxic, is in non-lethal doses the poorest depressant. The experiments do not of course serve to show the amount of depression at or near the death point, except in the cases of acetic and butyric acids, pH 5.0, Table VI. Since different acids are equally lethal at different hydrogen ion concentrations, it follows that the free hydrogen ions cannot be the chief factor in toxicity. The penetrating powers of the acid are probably of great importance and the nature of the anion or molecule may also be involved. Butyric and acetic acids which probably penetrate organisms the most readily of all the acids employed are also the most toxic. In a study of the toxicity of various acids for ciliate Protozoa, Collett ('19) reached the conclusion that hydrogen ion concentration is not the most important factor.

SUMMARY.

1. The effect of acidification of the medium on the rate of oxygen consumption of aquatic organisms was studied.

2. The acids used were: hydrochloric, nitric, sulphuric, carbonic, butyric, acetic, citric, and tartaric. They were added to water to produce acidities ranging from pH 7.5 to 5.0, at intervals of 0.5 pH.

3. *Planaria dorotocephala* was the chief animal used as material. Some tests with hydrochloric acid were also made using starfish and nudibranchs.

4. The acidification of natural waters, either salt or fresh, (pH 8.0), by any of the acids used except butyric causes a decrease in the rate of oxygen consumption at all acidities greater than pH 7.0. The majority of acids also cause depression between 7.8 and 7.0 but in the case of acetic acid and more doubtfully carbonic, there was some tendency towards a slight acceleration of the rate of oxygen consumption at these lower concentrations.

5. The decrease in the rate of oxygen consumption due to acids is completely and promptly reversible, as long as the animals are not actually injured.

6. The acidification of fresh water from which all carbonates have been previously removed has no or only a slight effect upon the rate of oxygen consumption of *Planaria*, except when the acidity is produced by carbon dioxide.

7. The depressing action of carbon dioxide is the same whether the gas is added to ordinary or to carbonate-free water.

8. From 6 and 7 it follows that the depressing action of acids in natural waters is due chiefly or wholly to the carbon dioxide which they liberate from the carbonates of such waters.

9. The depressing action of acidified natural waters on the rate of oxygen consumption of *Planaria* is not the same with different acids at the same hydrogen ion concentration. This appears to be due largely to the fact that the amount of carbon dioxide immediately liberated from the carbonates of the water differs with different acids at the same pH.

10. The depressing action of acids in natural waters is greater the greater the acidity up to an acidity of about pH 5.0. This is due to the fact that the more acid added, the greater is the quantity of carbon dioxide liberated.

11. Lower concentrations are, however, relatively more effective than higher ones.

12. The maximum amount of depression of oxygen consumption that can be induced by acids is about 50 per cent. This occurs at pH 4.0 to 5.0 and further acidification of the water does not increase the percentage of depression. At the acidity at which the maximum depression appears, the carbon dioxide content is about 3 per cent.

13. All of the carbonates of the fresh water employed are decomposed by acids at a pH of 4.0 to 5.0, producing a carbon dioxide concentration of 3 per cent. This might explain the facts given in 12 were it not that concentrations of carbon dioxide gas much higher than 3 per cent. do not increase the percentage of depression beyond 50 per cent., as long as the oxygen supply is ample. Concentrations of carbon dioxide gas up to 25 per cent. were tested.

14. From the facts cited in 13 it appears necessary to assume that the depression of the rate of oxygen consumption which can be induced by carbon dioxide does not exceed 50 per cent. as long as the oxygen supply is adequate.

15. A combination of high carbon dioxide content and low oxygen content practically abolishes the oxygen consumption of *Planaria*, even though the oxygen content used (2 cc. per liter) would be ample for normal respiration in the absence of carbon dioxide.

16. No explanation has been discovered for the differences between the action of carbon dioxide and other acids at low concentrations (pH 7.5 and 7.0).

17. Butyric acid has almost no action on the oxygen consumption of *Planaria*. No explanation has been discovered for this fact.

18. The different acids employed are lethal for *Planaria* in a given arbitrarily selected time (2 to 3 hours) at different hydrogen ion concentrations. The order of toxicity and the hydrogen ion concentrations at which the acids are equally lethal are: butyric (5.0), acetic (4.4), tartaric (3.6), citric (3.4), sulphuric (3.2) and nitric and hydrochloric (3.0).

19. The facts given in 18 prove that the hydrogen ion is not the cause of death but either the anion or the molecule of the acid is involved. Penetrability is probably also a factor. Death in acids appears to be due to coagulation.

20. The order of toxicity of the acids and the pH at which they are equally lethal are the same in ordinary and in carbonate-free water, showing that death is not due to carbon dioxide liberated.

21. Acidification of natural waters constitutes a method for depressing the rate of oxygen consumption of aquatic animals for experimental purposes.

22. The experiments herein presented cast doubt on the supposed importance of hydrogen ion concentration *per se* in biological processes.

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